PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION See Form PCT/IPEA/416 | |
|---|---|---|
| E31720 EKR/ANY | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/NO 2003/000335 | 07.10.2003 | 08.10.2002 |
| International Patent Classification (IPC) or national classification and IPC | | |
| C12N 5/08 | | |
| Applicant | | |
| Tjotta, Enok | | |

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 10 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 06.05.2004 | 26.01.2005 |
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INTERNATIONAL PRELIMI[redacted] REPORT ON PATENTABILITY

International Application No.
PCT/NO 2003/000335

**Box No. I Basis of the report**

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:

pages 1-148 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished

pages* _____ as amended (together with any statement) under Article 19

pages* 1-4 received by this Authority on 18-01-2005

pages* _____ received by this Authority on _____

☒ the drawings:

pages 1/65-65/65 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☒ the claims, Nos. 14, 15

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

**Supplemental Box**

**In case the space in any of the preceding boxes is not sufficient.**
Continuation of: Box I

The amendments filed with the letter dated 18-01-2005 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following:

The subject-matter of claims 14-15, i.e. that clonal stimulators may be used as medicaments for treatment of pathological conditions, has not been disclosed in the description. According to the Applicant, such applications of clonal stimulators are indicated on page 6, line 29 - page 7, line 2. However, this Preliminary Examining Authority does not consider such applications to be apparent from the text. The description only deals with the pathological properties of clonal stimulators, i.e. that they may be carcinogenetic or atherogenic, and that it is important to detect such compounds which may cause pathologically increased clonal growth.

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**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 12 (partially), 14, 15

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 12 (partially) are so unclear that no meaningful opinion could be formed *(specify)*:

Present claim 12 relates to the use of clonal mitotic inhibitors which are defined by reference to a desirable characteristic or property, namely that the inhibitors are detected by the screening method of claims 1-11. The claim covers all substances having this characteristic or property,

.../...

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 14, 15

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in the Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

**Supplemental Box**

**In case the space in any of the preceding boxes is not sufficient.**
Continuation of: Box III.2

whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such substances. In the present case, the claim so lacks support, and the application so lacks disclosure, that a meaningful examination over the whole of the claimed scope is impossible. Independent of the above reasoning, the claim also lacks clarity (Article 6 PCT). An attempt is made to define the substances by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful examination over the whole of the claimed scope impossible.

Consequently, the examination has been carried out for those parts of the claim which appear to be clear, supported and disclosed, namely those parts relating to the substances 4-OH-OPB, kolchicin, ibuprofen, naproxen, acetyl salicylic acid, p-hydroxy-azobenzene, 2-butyl-2-hydroxy-N-(4-hydroxy-phenyl)-N'-phenyl malonamide, piroxicam, 1,2-diphenyl-4-hydroxy-4-[2-(phenylsulfinyl)ethyl]-3,5-pyrazolidinedione and 4-hydroxy-4-aldehyde-1,2-diphenyl-pyrazolidinedione, which have been shown to have an inhibiting effect on clonal growth in low density area of cell gradient but no inhibiting effect in high density area of cell density.

Claim 12 relates partially to the treatment of diseases which are actually not well defined. The use of the definition "cloning in the immune system (e.g. primary immunological processes)" in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is not fully possible to determine the diseases for which protection might legitimately be sought. The lack of clarity is such as to render a meaningful complete examination not fully possible.

Consequently, the examination of claim 12 has been carried out for the defined pathological conditions, i.e. clonal growth in cancer, arteriosclerosis, autoimmunity, rejection of transplants, carcinogenetic and atherogenic processes and viral growth in cells.

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**Box No. V** **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-13 | YES |
| | Claims | | NO |
| Inventive step (IS) | Claims | 1-13 | YES |
| | Claims | | NO |
| Industrial applicability (IA) | Claims | 1-13 | YES |
| | Claims | | NO |

2. Citations and explanations (Rule 70.7)

This opinion is based on the set of amended claims filed on 18-01-2005.

As specified in Box III, claim 12 has been examined in respect of a limited number of substances which are considered to have support in the description and of which an inhibitory effect on clonal growth has been shown in experiments. Claim 12 has been examined in respect of the defined pathological conditions only.

Claims 14-15 have not been examined since the amendments are considered to go beyond the disclosure of the international application as filed. See also Box I and Supplemental Box.

Reference will be made to the following documents cited in the International Search Report:

D1) The Prostate, 32:115-121 (1997), Sven de Vos et al.
D2) Virology, 23:291-294 (1964), MacPherson et al.
D3) BIOSIS accession no. PREV198172018181, Carcinogenesis, 2:81-88 (1981), Baird et al.
D4) Journal of Clinical Hematology and Oncology, 12:67-76 (1982), Meischen et al.
D5) WO 0100585
D6) Gynecologic Oncology, 17:196-199 (1984), Weppelmann et al.
D7) Journal of Surgical Research, 54:7-11 (1993), Stoller et al.
D8) Atherosclerosis, 54:205-212 (1985), Bailey et al.

Present claims 1-11 relate to a three-step method of detecting agents which show specific inhibition or stimulation of clonal growth.

.../...

**Supplemental Box**

**In case the space in any of the preceding boxes is not sufficient.**
Continuation of: Box V

Documents D1-D4 represent the general state of the art.

The invention defined in claims 1-11 is not disclosed by any of these documents. The cited prior art does not give any indication that would lead a person skilled in the art to the claimed method. Therefore, the claimed invention is not obvious to a person skilled in the art.

Accordingly, the invention defined in claims 1-11 is novel and is considered to involve an inventive step. The invention is industrially applicable.

Present claims 12-13 relate to the use of clonal mitotic inhibitors detected by the method according to claims 1-11 for preparing a pharmaceutical preparation for the treatment of clonal growth in cancer, carcinogenesis from clonal growth of single cells, development of arteriosclerosis, autoimmunity, rejection of transplants, prophylaxis of carcinogenetic and atherosclerotic processes, cloning in the immune system and inhibiting viral growth in cells which are not densely collocated.

D5 discloses the use of hydroxy-protected 4-hydroxy 3,5-dioxo-pyrazolidines, e.g. 4-OH-OPB, for the manufacture of a medicament for the treatment of inflammatory or viral disease (e.g. HIV), autoimmune disease or tissue rejection, T cell tumours or Karposi's sarcoma (see claims 1, 7, 14, 24, 26 and page 9, last paragraph).

D5 does not discuss the use of the mentioned substances as clonal inhibitors in early phases of disease, before a development of collocated infected or immune cells takes place. Neither does D5 suggest a combined treatment intending to reduce the actual cell collocation sufficiently before using clonal inhibitors. Hence, D5 is considered to represent the general state of the art.

D6 describes the treatment with oxyphenbutazone and its positive effects on radiation therapy of carcinoma of the cervix (abstract and page 197, 2nd paragraph). Oxyphenbutazone

.../...

**Supplemental Box**

**In case the space in any of the preceding boxes is not sufficient.**
Continuation of: Box V

may be transformed into 4-OH-OPB in vitro, but according to the Applicant, this chemical reaction does not occur in vivo. Therefore, the effect of 4-OH-OPB is based on a mechanism that is completely different compared to the mechanism of oxyphenbutazone that is based on the inhibition of the prostaglandin synthesis. Thus, D6 is considered to represent the general state of the art.

D7 relates to the inhibition of the arachidonic acid cascade by use of phenylbutazone. Thoracic aortic and carotid artery cholesterol contents were decreased after treatment with phenylbutazone. The arachidonic acid cascade activates many inflammatory processes, and may also be involved in the pathogenesis of atherosclerosis (abstract). According to the Applicant, D7 shows that phenylbutazone affects the content of cholesterol in rabbits fed on cholesterol rich diet but has no effect on the growth of cell clones. 4-OH-OPB is shown to inhibit growth in single or a few cells that were not locally collocated and therefore there is not expected to be any effect on macroscopically developed arterial plaques or diffuse cholesterol infiltration in greater parts of the arterial wall. D7 is therefore not considered to be relevant for the present invention.

Document D8 represents the general state of the art.

Additional documents not cited in the International Search Report:
D9) WO 9604019
D10) WO 0033790

D9 discloses the use of a cytotoxin for the treatment of cell-proliferative diseases, e.g. atherosclerosis and lymphomas. The cytotoxin is for example colchicine, which acts by eliminating clonally expanded macrophages in mammalian tissue (see page 4, line 31 - page 5, line 19, claims 13-15 and 22).

D9 does not, however, take into consideration several factors which are central to the present invention, e.g. collocation inhibition of the clonal inhibitors and the ability to inhibit metastatic or local spread of tumours. Further, no selection specific and non-toxic clonal inhibitors has been made.

Therefore, D9 is considered to represent the general state of the art.

.../.../...

**Supplemental Box**

**In case the space in any of the preceding boxes is not sufficient.**
Continuation of: Box V

D10 relates to a method of preventing development of cardiovascular disease in an individual having a region of nonproliferating and proliferating cells, the method comprising administering to the individual an effective amount of a non-steroidal anti-inflammatory drug (NSAID) to induce apoptosis of the proliferating cells. The NSAID may be aspirin (i.e. acetyl salicylic acid), indomethacin ibuprofen, naproxen or piroxicam. The cardiovascular disease is for example atherosclerosis.

In D10, specific clonal inhibitors have not been selected, which may result in inclusion of stimulators of clonal growth or toxic substances which inhibit both collocated cells, organs and the whole organism. Further, ignoring thefact that collocated cells might abrogate the positive effect may result in ineffective therapy.

Hence, D10 is considered to represent the general state of the art.

The subject-matter of claims 12-13 is novel and is considered to fulfil the requirements of inventive step and industrial applicability.

**Box No. VIII Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The present claims 1 and 3-11 relate to a test method performed in vivo on an animal. As the claims are drafted, the claims do not exclude the animal from being human. Such subject matter is not allowable in some countries.

Claim 9 should only refer to claims relating to the in vivo method or should be amended to include an in vitro alternative to paragraph (a).

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PCT/NO2003/000335
18-01-2005
JC13 Rec'd PCT/PTO 06 APR 2005

Claims

1.

Method for *in vivo* tests in animals by the detection of agents showing specific inhibition or stimulation of clonal growth by selection and testing of drugs, potential drugs, food, food additives, toxins, potential toxins, components from physiological or pathological processes, including microbes and the effect of physical stimulation (e.g. radioactivity or ultrasound) on the body or parts of the body with specific anti-clone or clone stimulating effects comprising:

a) a clonal test for studying the influence of these agents on cloning;
b) a test detecting the degree of neutralization of inhibiting effect of local collocation of cells for studying for example how increase of local cell concentration can reduce or in other ways change the effect of the mentioned substances or physical effects on the process of cloning and on the toxicity and;
c) tests for influencing the development of metastases in other ways than through cloning, for instance by the influence of the mentioned substances or principles on export or liberation of metastasising cells from a malignant tumour or localization where tumour cells are located.

2.

Method for *in vitro* tests in animals by the detection of agents showing specific inhibition or stimulation of clonal growth by selection and testing of drugs, potential drugs, food, food additives, toxins, potential toxins, components from physiological or pathological processes, including microbes and the effect of physical stimulation (e.g. radioactivity or ultrasound) on the body or parts of the body with specific anti-clone or clone stimulating effects comprising:

a) a test for studying the influence of these agents on cloning;
b) a test detecting the inhibiting effect of local collocation of cells for studying for example how increase of local cell concentration or collocation can reduce or in other ways change the effect of the mentioned substances or physical effects on the process of cloning and on the toxicity and;
c) tests for influencing the development of metastases in other ways than through cloning, for instance in tissue cultures by the influence of the mentioned substances or principles on liberated single cells, that were analogous to metastasising cells, outside a localization containing collocated cells of the same kind.

3.
The method according to claim 1 or 2 wherein said cloning test comprises:

a) seeding of cells in agar with or without special growth factor(s):
b) preparing or incubation of special gels;
c) incubating in suitable temperature and atmosphere and;
d) follow up of cells and development of clones.

4.
The method according to anyone of claims 1-3 wherein the method is being performed by using clonal test in fluid medium, for instance in plates with wells.

5.
The method according to anyone of claims 1-4 wherein the mentioned cells comprise: malignant cells, normal cells, cell lines, transformed cells and cells from the tumour or malignant disease of the patient, or cells from the immune system that are clone selected after immunization where the latter can be detected and quantified.

6.
The method according to anyone of claims 1-5 wherein the mentioned cells being a cell line, BHK21/c13 or BHK21/C13 cells transformed with polyoma virus.

7.
The method according to anyone of claims 1-6 wherein the mentioned growth factor(s) comprises insulin, serum, insulin like growth factors, cytokines, or serum extenders and conditioned medium or a combination of these.

8.
The method according to anyone of claims 1-7 wherein the mentioned test for neutralizing the effect by cells in locally high density comprises:

a) transplantation of tumour cell(s) to an animal, for instance Ehrlich ascites cells transplanted to mice or seeding experimental cell cultures with cells in claim 5;
b) adding to the test substances mentioned in claim 1 or 2, or giving them to the test animal;

c) follow up the tumour cells in the animal or the cells in experimental cell cultures.

9.
The method according to anyone or more of claims 1-8 wherein the said tests for influencing the development of metastases comprise:

a) injection of tumour cell(s) in the animals for testing the ability to develop metastases ascites or local tumours;
b) applying the agent(s) mentioned in claim 1 or 2 and;
c) follow up the ability that the substance(s) has (have) to affect the liberation of cells, migration, and the ability to form local tumour.

10.
The method according to claim 9 wherein the said tumour cells being transplanted Ehrlich carcinoma cells.

11.
The method according to anyone of the claims 1-10 wherein said method detects compounds causing increased number of clones and/or facilitates the growth and migration of metastases and/or growth of primary tumours.

12.
Use of clonal mitotic inhibitors detected by the method according to anyone of claims 1-11 for preparing a pharmaceutical preparation for the treatment or prophylactic of clonal growth in cancer including development of clones resistant to treatment, carcinogenesis from clonal growth of single cells without or following irradiation or other physical effects (e.g. ultrasound liberating growth factors as insulin like growth factors that would stimulate clonal growth), development of arteriosclerosis, autoimmunity, rejection of transplants or or prophylaxis of carcinogenetic and atherosclerotic processes and cloning in the immune system (e.g primary immunological processes), and also inhibiting viral growth in cells not densely collocated.

13.
The use according to claim 12 wherein the clonal mitotic inhibitors comprising 4-OH-OPB, Kolchicin, Ibuprofen, Naproxen, Acetyl salicylic acid, p-hydroxy-azobenzene, 2-

Butyl-2-hydroxy-N-(4-hydroxy-phenyl)-N'-phenyl malonamide, 1,2-diphenyl-4-hydroxy-4-[2-(phenylsulfinyl)ethyl]-3,5-pyrazolidinedione or analogues thereof.

14.
The use of clonal mitotic stimulators according to anyone of the claims 1-11 for preparing a pharmaceutical preparation for treatment or prophylaxis of pathological conditions associated with decreased clonal activity or clonal growth (e.g. immunological failure) or where increased clonal growth is desired (e.g. at bone marrow transplantation).

15.
The use according to claim 14 where the clonal mitotic stimulators comprise insulin, insulin like growth factors, conditioned medium, serum factors or serum extenders (e.g. Mito+), Diclofenak, Sulindak or Benzo(a)pyrene or analogues of these.